Improvement of Penetrability of Sugi by Treatment with Pectinase

Makoto OHKOSHI, Toshimitsu HATA, Kanji NAKATO and Takeshi SADOH

スギ生材のペクチナーゼ処理による浸透性の向上

大越　誠・畑　俊充・中戸　莞二・佐道　健

Abstract

Green sapwood, intermediate wood and heartwood of Sugi were treated with pectinase in order to improve their penetrability which is depressed by pit aspiration during wood drying. The penetrability of sapwood specimen increased owing to the preferential attacks of enzyme against tori of pit membranes. The damaged tori were observed in the treated sapwood under SEM. The penetrability of intermediate wood and heartwood specimen did not increase by the treatment with pectinase, possibly because the wood extractives prevented the enzyme contacting with the pectic substances in tori. The mechanical properties of specimen did not significantly decrease by the treatment.

要　旨

仮道管内こう相互を直列につなぐ有縁壁孔は，径が微小であり，壁孔閉鎖を生じることなどによって，針葉樹材中の物質の流動を支配する。壁孔膜のトールスがペクチン質を多く含むことに着目して，スギ生材をペクチナーゼで処理し，浸透性の向上をはかった。

処理により辺材の浸透性は増加し，壁孔閉鎖をほとんど生じない凍結乾燥を行なった辺材の浸透性に近い値を示した。処理を行なった辺材では，トールスの破壊がSEMにより観察され，試験体の力学的性質の低下が認められなかったことから，この増加は酵素によるトールスの選択的な分解によることが明らかである。しかし，処理による浸透性の向上には試験体間でかなりの変動がみられた。これは，トールスにおけるペクチン質の組成および抽出成分量の変動によると考えられる。移行材および心材では浸透性は向上しなかった。これは，トールスに沈着した抽出成分がペクチン質への酵素の接触を妨げたためと考えられる。

Introduction

Main paths of fluid flow in softwoods are capillaries of tracheid lumina and bordered pits

This report was presented at the 35th Annual Meeting of the Japan Wood Research Society at Tokyo in April, 1985.

connected in series. The bordered pits control mainly the flow of fluids, because they have small pores and are subject to aspiration during the wood drying and the heartwood formation. Experiments in improvement of the penetrability of wood have been carried out with the various methods, e.g. steaming[1], solvent extraction[2], degradation by micro-organisms[3] and pretreatment with enzymes.[4] Tori of the pit membranes contain pectic substances in addition to cellulose and hemicellulose.[5] To remark on that, we tried to degrade the tori by attacks of pectolytic enzyme in order to increase the penetrability of wood. Longitudinal penetration rates of water through the treated wood specimens were measured and were compared with those of the untreated and the freeze-dried specimens in which pit aspiration was avoided. The effect of wood extractives on penetrability was examined by measuring the penetration rate of the specimen extracted with ethanol-benzene mixture. The tori of the treated specimens were observed with a scanning electron microscope (SEM). Changes in the mechanical properties of wood by the pectinase treatment were also measured.

Experimental

1. Material and enzyme used

Wood: A twenty-three-year-old Sugi [*Cryptomeria japonica* D. Don] tree was freshly felled, and was cross-cut at various height from 0.3 to 7.5 meters. The logs were then quartersawn. For each quadrant, specimens of dimension of 0.8 cm (R) × 0.8 cm (T) × 15 cm (L) were obtained from outer and inner sapwood, intermediate wood and heartwood. The specimens were stored at −20°C in never-dry state until beginning the experiment.

Enzyme: Enzyme used was a commercial preparation of pectinase (Sankyo Ltd., Sclase N). The pectinase contains pectinesterase (PE) and polygalacturonase (PG).[6] Pectic substances, which are the substrates of pectinase, consist of pectic acid and pectin in which the carboxyl groups are partly esterified. The PE hydrolyzes a methyl-ester in pectin. The PG hydrolyzes an α-1,4 linkage in pectic acid. Therefore, the highly esterified pectin is cleaved in a two-stage reaction by PE and PG. The most suitable conditions for pectinase activity are temperature of 40–45°C and pH value of 2.5–5.0.[7]

2. Methods of treatment

The specimens of outer and inner sapwood, intermediate wood and heartwood were treated by following methods. A set of eleven to fifteen specimens was used for each treatment.

Air-drying and freeze-drying: Two sets were used for this series. A set of specimens stored at −20°C was immediately dried at 45°C and the other set of specimens was freeze-dried in a vacuum at −10°C. It is expected that the freeze-drying prevents pit aspirations in the sapwood.[8]

Pectinase treatment: Some other sets of specimens were thawed, and then were treated with buffer or pectinase by the following procedure. The enzyme was used with a sodium acetate buffer (pH 4.1) containing 0.15% sodium benzoate as fungicide. The concentrations of pectinase solution used were 0.2 and 1%. The sapwood specimens were soaked in the buffer or the pectinase solution. The intermediate wood and the heartwood specimens were impregnated with the solutions after evacuation. The specimens immersed into the solutions

were subjected to supersonic waves (28 kHz, 600 W) for 2 to 18 hours to promote the impregnation. The solutions were maintained at 40°C during the supersonic wave treatment. Subsequently, the specimens were placed in the solutions for 3 or 6 days at 40°C. After removal from the solutions, the specimens were again subjected to the supersonic waves for 90 minutes in fresh water. Destruction of the tori which had become fragile by enzyme attacks could be expected by this treatment. The specimens were then dried at 45°C.

Extraction: The last set of specimens was extracted with an ethanol-benzene mixture by the following way. After thawing the specimens, water in the specimens was replaced with ethanol followed by the ethanol-benzene mixture. The specimens were extracted in a soxhlet apparatus for 2 to 5 weeks. After extraction, the mixture in the specimens was replaced with ethanol and next water. The specimens were then dried at 45°C.

3. Measurements of penetrability and mechanical properties, and SEM observation

All specimens were conditioned at a room of 20°C and 60%RH. Four sides of the specimens were coated with vinyl chloride resin in order to prevent the capillary rise of water along the wood surface, as reported previously.[9] One end of the specimens was dipped in water, allowing for water to penetrate longitudinally into the specimens. The penetration weight of water was measured at certain intervals. After the penetration measurement, the specimens were dried and conditioned. The specimens were then tested in static bending at a span of 11 cm. Modulus of elasticity, strength and work to maximum load per unit volume of the

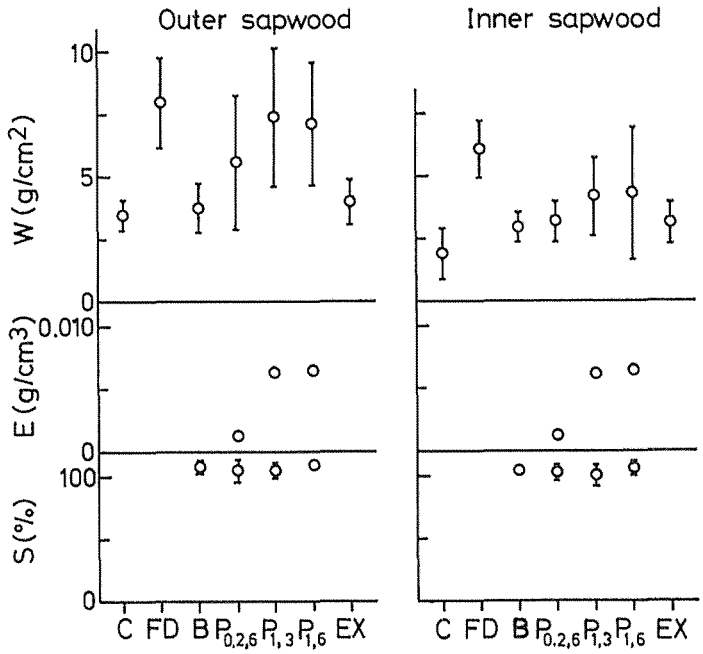


Fig. 1 Effects of different treatments on the penetrability of sapwood.
Notes: *S*: ratio of the weight of solutions absorbed into specimen to the saturation, *E*: mass of enzyme taken up in specimen, *W*: penetration weight of water after 40 minutes, C: untreated, FD: freeze-dried, B: treated with buffer, P: treated with pectinase (first subscript: concentration of pectinase, second subscript: days of treatment), EX: solvent extracted.

specimen were calculated from a load-deflection curve. After the test, the specimens were radially split. The pit membranes were observed under SEM (Hitachi S-500).

Results and Discussion

1. Treatability of sapwood with pectinase

Results of the treatments of sapwood are shown in Fig. 1, where S(%) is ratio of the weight of solutions absorbed into specimen to the saturation which is estimated from specific gravity of specimen, E (g/cm^3) a mass of enzyme taken up per unit volume of specimen, and W (g/cm^2) a penetration weight of water per unit sectional area of specimen after 40 minutes. Averages and ranges of standard deviation of those are shown in Fig. 1. The values of S for the specimens treated with buffer (B) and pectinase (P) were about 100% in both outer and inner sapwoods, indicating that the specimens have been saturated with those solutions. There were fivefold differences in the E value between 0.2 and 1% pectinase treated specimens in proportion to the concentration of solution.

In the untreated kiln-dried specimens (C), the outer sapwood was more permeable than the inner one. In the freeze-dried specimens (FD) in which the pits were expected to be unaspirated, the W value was twice to three times as much as in the untreated. This indicates that the pits mainly control the penetrability of specimen. There was little difference in W between the untreated and the buffer treated specimens. It is considered that the acid buffer of pH 4.1 brought about little change of penetrability of wood and that external micro-organisms could not work during the incubation owing to the fungicide. The W value for the extracted specimens (EX) did not differ from that for the untreated, indicating that wood

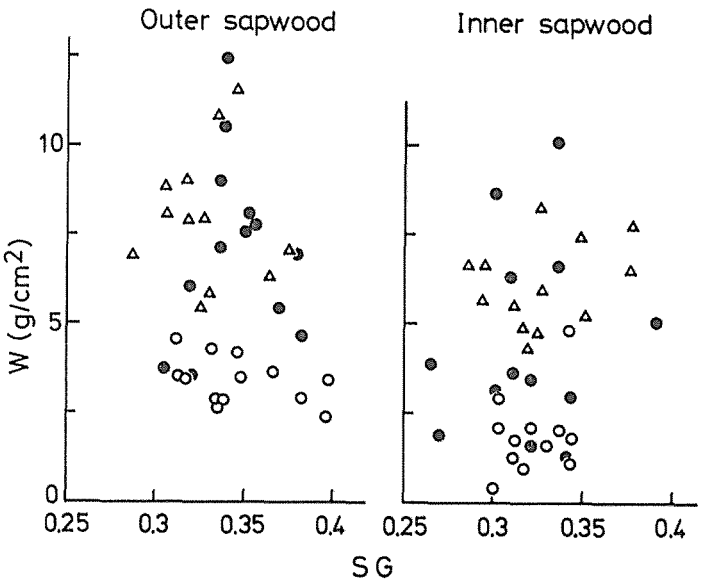


Fig. 2 Relations between the penetration weight (W) and the air-dry specific gravity (SG) of sapwood specimen.
Notes: ○: untreated, ●: treated with 1% pectinase for 6 days, △: freeze-dried.

extractives had no influence on the penetrability of sapwood.

The penetrability of sapwood specimens increased by pectinase treatment. As the buffer did not change the penetrability of specimens, those increases resulted from the enzyme attacks. The outer sapwood was more effectively treated with pectinase than the inner one. In the outer sapwood, the W values for the 1% pectinase treated specimens were as much as the freeze-dried specimens. Differences in the W value between 0.2 and 1% pectinase treated specimens were relatively small, though there was fivefold difference in the E value. The S values were about 100% for the specimens treated with pectinase solutions of both concentrations as mentioned above. Therefore, the sapwood specimens would be effectively treated on condition of the saturation with pectinase solutions in spite of the concentration. For the specimens treated with 1% pectinase, there was no difference in W between 3 and 6 day treatment. This indicates that the 3 day reaction of pectinase was sufficient to increase the penetrability of specimen.

An effect of the pectinase treatment on the penetrability was varied among the specimens

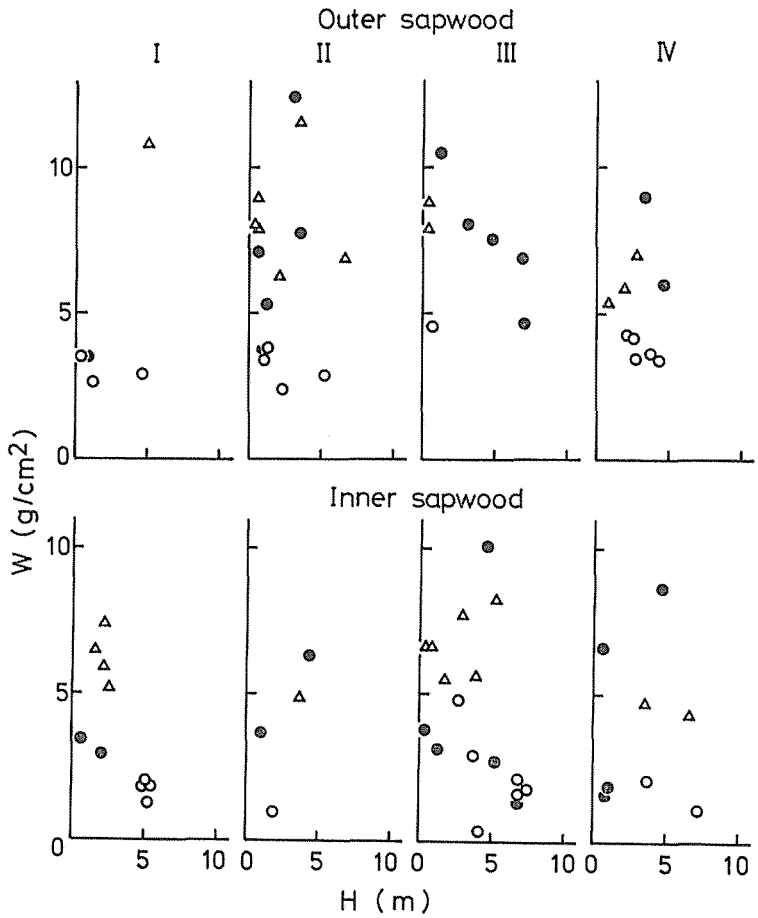


Fig. 3 Changes of the penetration weight (W) with the height (H) and the quadrantal positions (I, II, III, IV) in tree.
Notes: Symbols are same as shown in Fig. 2.

treated by the same concentrations and duration. The variations of penetrability, which were indicated by standard deviation of W, were greater in the pectinase treated specimens than in the untreated. Figure 1 shows that E and S were less variable among the specimens than W. This result indicates that the variations of E and S do not affect the variation of treatability. An effect of the specific gravity of specimen on the treatability with pectinase was examined. Results are shown in Fig. 2. There were no correlations between W and the air-dry specific gravity in the 1% pectinase treated specimens as well as in the untreated and the freeze-dried specimens. This indicates that the treatability with pectinase had no relation with the specific gravity, or the void volume of specimen. Next, an influence of the location in tree, where the specimens were taken, on the treatability was examined. Changes of W with the location in tree are shown in Fig. 3. In each treatment, the W value varied regardless of the locations. As a result, the specific gravity and the location in tree did not affect the variation of treatability with pectinase.

It is proposed that the variation of treatability was due to the differences of the chemical compositions among tori of pit membranes, i.e., the variety of pectic substances and the amount of wood extractives in tori. The difference of treatability between the outer and the inner sapwood may have been also derived from them. The pectinase is unable to efficiently degrade the highly esterified pectic substances because of the two-stage reaction by PE and PG. As mentioned above, the wood extractives had no influence on the penetrability in the sapwood. On the other hand, Morishita et al.[10] showed that the wood extractives dissolved in buffer did not decrease the activity of enzyme. The extractives in tori, however, are considered to prevent the enzyme contacting with the pectic substances.

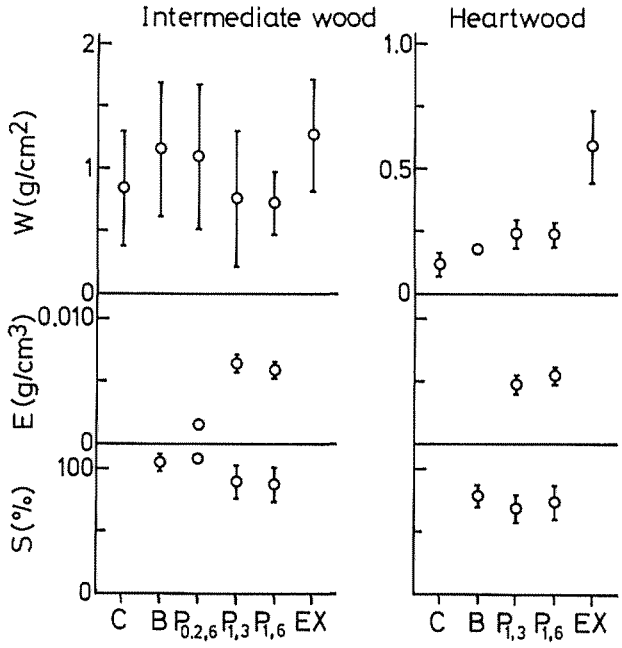


Fig. 4 Effects of different treatments on the penetrability of intermediate wood and heartwood.
Notes: Abbreviations are same as shown in Fig. 1.

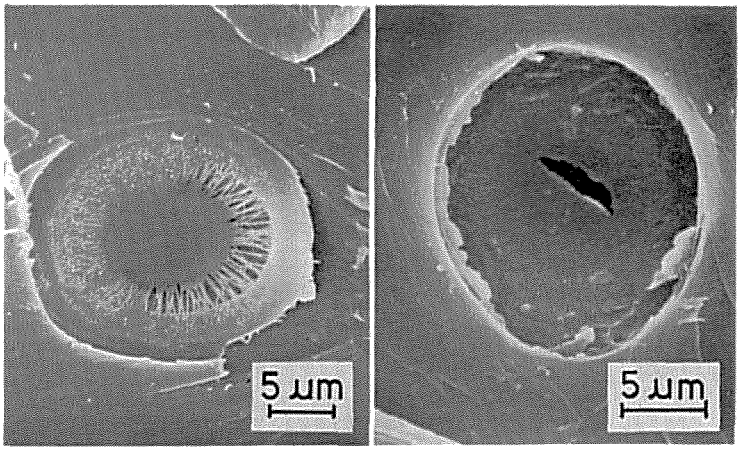
2. Treatability of intermediate wood and heartwood with pectinase

Results of the treatments of intermediate wood and heartwood are shown in Fig. 4. Note that ordinate scales of W are different from those in Fig. 1. The penetrability of intermediate wood and heartwood was much less than the sapwood owing to the pit aspiration during heartwood formation. In the intermediate wood the penetrability of specimen did not increase by the pectinase treatments, though S and E of the specimen were as much as in the sapwood. The aspiration of pit may have been one of the reasons why the pectinase treatment was not effective. There was little difference in W between the untreated and the extracted specimens, indicating that the wood extractives did not affect the penetrability. It is, however, supposed that the wood extractives in tori prevented the enzyme contacting with the pectic substances in the intermediate wood like the sapwood.

In the heartwood, the W value for the extracted specimen was about five times as much as the untreated specimen. This indicates that the wood extractives was another factor for the lower penetrability of heartwood. The S value for the pectinase treated specimen was less than 100% owing to the lower penetrability of specimen. The W value increased slightly by the pectinase treatment. However, the penetrability of the treated heartwood specimen was very small in comparison with that of the sapwood. As mentioned above, W did not increase by the pectinase treatments in the intermediate wood, though the specimen was almost saturated. Therefore, it cannot be considered that the lower S in the heartwood was the factor for the less effect of pectinase treatment. It is supposed that the wood extractives in tori prevented the enzyme contacting with the pectic substances.

3. Change in the appearance of pit membrane by pectinase treatment

Pit membranes in the untreated and the pectinase treated sapwood specimens were observed with SEM. Figure 5 shows the electron micrographs of the membranes. Tori in the untreated specimens are flawless as shown in Fig. 5(a). The damaged tori were found in the pectinase treated specimens, as shown in Fig. 5(b). This means that the tori which had become fragile by the enzyme attacks against pectic substances were destroyed by either



(a) (b)

Fig. 5 Electron micrographs of pit membranes in sapwood.
Notes: (a): untreated, (b): treated with 1% pectinase for 6 days.

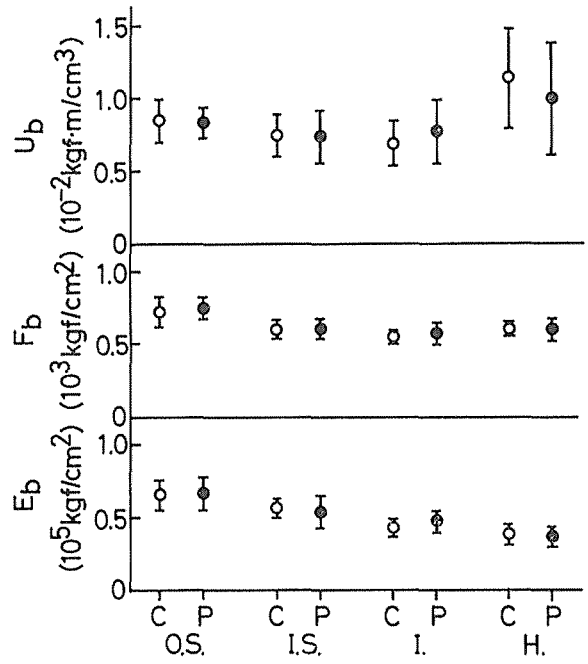


Fig. 6 Modulus of elasticity (E_b), strength (F_b) and work to maximum load (U_b) in bending of the untreated specimen and the specimen treated with 1% pectinase for 6 days.
Notes: O.S.: outer sapwood, I.S.: inner sapwood, I.: intermediate wood, H.: heartwood, Other abbreviations are same as shown in Fig. 1.

supersonic irradiation or drying. In the treated specimens, some flawless tori were found together with the damaged tori. This suggests that an activity of pectinase was different among the tori. Some of pits with the damaged tori may not aspirate during the drying due to low capillary force of water corresponding to the large pore in tori. Anyway, it is concluded that such pore in the damaged tori facilitated the penetration of water through sapwood.

4. Effect of the pectinase treatment on the mechanical properties of specimens

Results of the static bending test are shown in Fig. 6. Modulus of elasticity (E_b), strength (F_b) and work to maximum load per unit volume of the specimen (U_b) did not differ significantly between the untreated and the pectinase treated specimens. This means that the pectinase did not work on the main components which support the cell wall mechanically. The pectinase preferentially attacked against the pectic substances in tori to improve the penetrability of specimen.

Conclusion

Green Sugi specimens were treated with pectinase. The treatment increased the penetrability of sapwood without the decrease in the mechanical properties. The penetrability of the treated specimen was as much as that of the freeze-dried specimen. It was concluded that the increase in penetrability was derived from the preferential attacks of enyzme against tori in pit membranes. The effect of the treatment was varied among the specimens. Prob-

ably the variety of composition of pectic substances and the difference of amount of wood extractives among tori are the factors of the variation. The penetrability of intermediate wood and heartwood did not increase by the treatment with pectinase. It was supposed that the wood extractives in tori prevented the enzyme contacting with the pectic substances. This means that the pre-extraction of the wood may improve the effect of pectinase treatment.

Acknowledgment

The authors are deeply grateful to Dr. Yuji Imamura, Wood Research Institute, Kyoto University, for his advice and kind assistance in SEM observation. This work was supported financially by a Grant-in-Aid for Special Project Research (59127037) from the Ministry of Education, Japan.

References

1) Erickson, H. D., Crawford, R. J.: The effects of several seasoning methods on the permeability of wood to liquids. *Proc. Am. Wood Preserv. Assoc.*, **55**, 210–220 (1959)
2) Koran, Z.: Air permeability and creosote retention of Douglas-fir. *Forest Prod. J.*, **14**, 159–166 (1964)
3) Johnson, B. R., Gjovik, L. R.: Effect of *Trichoderma viride* and a contamination bacterium on microstructure and permeability of loblolly pine and Douglas-fir. *Proc. Am. Wood Preserv. Assoc.*, **66**, 234–240 (1970)
4) Adolf, F. P.: Untersuchungen zum Tränkverhalten von enzymatisch vorbehandeltem Nadelholz. *Holz als Roh- und Werkstoff*, **34**, 163–166 (1976)
5) Nicholas, D. D., Thomas, R. J.: The influence of enzymes on the structure and permeability of loblolly pine. *Proc. Am. Wood Preserv. Assoc.*, **64**, 70–76 (1968)
6) Nakahama, T.: Pektolytische Enzyme. *Scientific Reports of Kyoto Prefectural Univ., Agriculture*, **20**, 83–101 (1968)
7) Sankyo Ltd.: Sclase N Pamphlet
8) Thomas, R. J.: The ultrastructure of southern pine bordered pit membranes as revealed by specialized drying techniques. *Wood and Fiber*, **1**, 110–123 (1969)
9) Yamamoto, K., Ohkoshi, M., Nakato, K., Sadoh, T.: Natural penetration of water into softwoods—Analysis by soft X-ray densitometry. *J. Soc. Materials Science*, **32**, 869–874 (1983)
10) Morishita, S., Nakato, K., Sadoh, T.: Preprint of the 35th Annual Meeting of the Japan Wood Research Society, Tokyo, Apr. 1985, p. 55